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A NEW CLASS OF CLAY POLYMER COMPOSITE FILMS AS SEED COATING MATERIAL FOR THE EXTENDED RELEASE OF MICRONUTRIENT, Fe^{+2}

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ABSTRACT

The world demand for fertilizer in general is increasing day by day. Surface feeding is the most common practice of providing micronutrients to the growing plants. In spite of its popularity and widespread use, the method suffers from disadvantages; excessive use of micronutrients and decreased efficiency of nutrient utilization. Therefore, there is an urgent need to prevent the excessive use and waste of micronutrients. Micronutrients are required in the early stage (2-3 weeks) of the plant's growth; hence, it is desirable that micronutrients are made available in the vicinity of the seedlings and growing plants. In order to overcome these drawbacks attempts has been made to design a micronutrient (Fe^{+2}) containing biodegradable polymeric film as a seed coating material to provide an extended release of Fe^{+2} in the immediate vicinity of the seed when it is sown in the soil.

The synthesized films have been characterized by XRD, FTIR and TGA technique, since; water constitutes a basic requirement for germination. Therefore, barrier properties of films have also been investigated. It has been found that the Mt starch glycerol composite film attains 79% swelling capacity in water within 28 hours. The film also possesses good pore volume and water vapor permeability. The time dependent cumulative release of Fe^{+2} in an aqueous soil extract was monitored for the starch glycerol film and clay (Montmorillonite, Bentonite and Vermiculite) starch glycerol composite films. Mt starch glycerol composite film was found to be the best with76% of the cumulative release of Fe^{+2} in an extended manner for15 days. This Fe^{+2} containing Mt starch glycerol composite film was used for coating wheat seeds. The coated and uncoated seeds were sown in normal and deficient soils, results indicate better crop production from the coated seeds. This work will be published separately.

KEYWORDS: *Seed Coating, Micronutrients, Extended Release of Micronutrient & Montmorillonite*

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Original Article

INTRODUCTION

Nutrient content of a seed must be adequate to sustain the early growth of a plant until the root system can provide nutrients from the soil to the plant (1). There are seventeen nutrients that are essential for the healthy growth of plants. They are divided into two main groups: mineral and non-mineral nutrients (2-3). The non-mineral nutrients are hydrogen, oxygen and carbon. These nutrients are obtained from air and water (2). The fourteen mineral nutrients are divided into two groups, macro and micro nutrients. Relatively large amounts of essential mineral elements are required by plants for plant growth and are called macronutrients. Macronutrients are further subdivided into primary and secondary nutrients.

The primary nutrients (N, P, and K) are required to be supplemented in the soil as plants use them in large

amount for their growth and survival. The secondary nutrients (Ca, Mg, S) are sufficient in the soil so these are very often does not require to be supplemented.

Micronutrients, belonging to the mineral nutrient category, are required in relatively smaller quantities. These micronutrients are very important for the growth of plants if any element is lacking in the soil or not adequately balanced with other nutrients, growth suppression or even complete inhibition may result (4-5). During the germination period of the seed, sprouting and initial growth of the plant is particularly critical because the roots and stem of the growing plant are small and very soft. Even a small amount of damage can kill the entire plant. Therefore, micronutrients are required in early stage (2-3 weeks) of plant growth and therefore, must be available in the seed or in the vicinity of the seed (6). Micronutrients activate enzymes that are responsible for the synthesis of proteins. These are used for chlorophyll and carbohydrate formation. These are essential in the formation of auxin, which help in growth regulation, stem elongation and flowering. Micronutrients are required for healthy plant cell formation (3, 7). Micronutrient deficiencies are widespread. Thirty percent of global cultivated soil is deficient with low iron availability, because of an excessive alkalinity of the soil (pH> 6.5). Iron is an essential micronutrient for almost all living organisms because it plays an important role in metabolic processes such as DNA synthesis, respiration and photosynthesis. Fe deficiency reduces chlorophyll production, as identified by interveinal chlorosis with a sharp distinction between the veins and chlorotic areas in young leaves (8-9).

Thus, there is an urgent need to take care of iron deficiency. The conventional method for providing micronutrients to the plant is by soil feeding. In soil feeding method, nutrient is applied to the surface or the entire field. This method is easy and nutrients are immediately provided to the plants, but it involves non-uniform distribution and causes high nutrient loss. High level of micronutrients can be toxic for crops and low level of micronutrients may results deficiency in the crops. Seed coating is the modified method which can provide micronutrients in sustained or controlled manner. At present, micronutrients are applied mainly as slurry to the seeds and then dried. The disadvantage of this method is that it is difficult to get a uniform coating around the seed and higher amount of material may be rubbed off during transportation and handling.

Therefore, to overcome these problems there has been a need of biodegradable micronutrient/s encapsulated seed coating film. This composite film creates a nutritious, environment in the immediate vicinity of the germinating seed. This provides a "boost" for the seedling in its critical early stages of development. This is environmentally friendly and economical for farmers because it prevents excessive use of fertilizers.

EXPERIMENTAL

Materials

Starch (procured from central drug house Pvt. Ltd. New Delhi, India), glycerol (procured from Glaxo India Ltd., Mumbai, India), $FeSO_4$ (procured from Thomas baker chemicals Pvt. Ltd. Mumbai, India) and Montmorillonite, Bentonite and Vermiculite clays were obtained from Sigma Aldrich, St. Louis, MO, U. S. A.

Synthesis of Seed Coating Materials

Seed coating film/s ideally should be biodegradable with good barrier and mechanical properties. Keeping this in mind, starch was selected as the material of choice and glycerol was used as a plasticizer for better film formation.

Synthesis of Starch Glycerol Film

Keeping the amount of starch constant (500mg) the amount of glycerol was varied from 50 µl to 250 µl and series of experiments were performed, table 1. A series of 250ml Teflon beakers containing 500mg of starch and 25ml of double distilled water was taken and the mixture was stirred for one hour at 25 ºC followed by the addition of 150 µl of glycerol while maintaining the stirring. After the complete addition of glycerol the mixture was heated to 80 ºC till a homogeneous viscous solution was obtained. Each solution was poured on separate petri dishes of equal sizes and was air dried at 25 ºC for 48 hours.

Table 1: Effect of Glycerol on Film Formation

Sample Code	Starch (mg)	Glycerol (µl)	Observation
F: Fe - 01	500	50	Film formation did not take place
F: Fe – 02	500	100	Film formation did not take place
F: Fe – 03	500	150	Film formation took place
F: Fe – 04	500	200	Film formation did not take place
F: Fe – 05	500	250	Film formation did not take place

Synthesis of Fe^{+2} Starch Glycerol Film

Varying amount of ferrous sulfate (500µg to 3000µg of Fe^{+2}) was added to a series of 250ml Teflon beakers containing 25 ml of double distilled water to these beakers 500mg of starch powder was added, the mixture was stirred for one hour at 25 ºC on an electric hot plate cum stirrer followed by the addition of 150 µl of glycerol while maintaining the stirring, after the complete addition of glycerol the mixture was heated to 80 ºC till a homogeneous viscous solution was obtained. These solutions were poured into a number of petri dishes of same size and were air dried at 25 ºC for 48 hrs. Films thus formed were peeled off from each petri dish.

Synthesis of Mt Starch Glycerol Composite Film

Varying amount of Mt (20mg to 100mg, table 3) was added to a series of 250ml Teflon beakers containing 25 ml of double distilled water and the mixtures were stirred for one hour at 25 ºC on an electric hot plate cum stirrer. 500mg of starch was added in each beaker and the mixtures were stirred for one hour at 25 ºC on an electric hot plate cum stirrer followed by the addition of 150µl of glycerol while maintaining the stirring, after the complete addition of glycerol the mixture was heated to 80 ºC till a homogeneous viscous solution was obtained (11-13). These solutions were poured into a number of petri dishes and were air dried at 25 ºC for 48 hours. Films thus formed were peeled off from each petri dish.

Synthesis of Fe^{+2} Mt Starch Glycerol Composite Film

Fe^{+2} Mt starch glycerol composite films were synthesized by the following two methods:

- A series of 250ml Teflon beakers containing 500mg of starch and 2500µg of Fe^{+2} in 25ml of double distilled water was taken and the mixture was stirred for one hour at 25 ºC to these solutions varying amounts of Mt (20mg to 100mg, table 3) was added followed by the addition of 150 µl of glycerol, in each beaker, while maintaining the stirring. After the complete addition of glycerol the mixture was heated to 80 ºC till a homogeneous viscous solution was obtained. Each solution was poured on separate petri dishes of equal sizes and was air dried at 25 ºC for 48 hours.

- A series of 250ml Teflon beakers containing varying amounts of Mt (20mg to 100mg, table 3) in 25 ml of an aqueous solution of ferrous sulfate containing 2500µg Fe^{+2} were stirred for one hour at 25 ºC on an electric hot plate cum stirrer. To these Mt containing ferrous sulfate solutions 500mg of starch was added and the mixtures were stirred for one hour at 25 ºC on an electric hot plate cum stirrer followed by the addition of glycerol while maintaining the stirring, after the complete addition of glycerol the mixture was heated to 80 ºC till a homogeneous viscous solution was obtained. These solutions were poured into a number of petri dishes and were air dried at 25 ºC for 48 hrs. Films thus formed were peeled off from each petri dish.

RESULTS AND DISCUSSIONS

Characterization

X-ray Diffraction (XRD) Studies

X-ray diffraction patterns were recorded on a Philips X′ Pert-PRO MRD D8 Discover Bruker AXS and PAN analytical B.V., Lelyweg 1, 7602, EA ALMELO, The Netherlands operating at 50 KV, Cu Kα line (n =1 Å) was used in the present case.

X-ray diffraction patterns of Mt, Mt Fe complex, Fe^{+2}Mtstarchglycerol composite films are represented (figure 1). In Mt Fe Complex, the characteristic diffraction peak of Mt was observed to be shifted from 6.2º to 5.8° after interaction with Fe^{+2} resulting in an expansion of the interlayer region by 0.94Å indicating probable intercalation of Fe^{+2} in to the Mt gallery (14). In case of Fe^{+2} Mt starch glycerol composite film an interlayer expansion of 19.34 Å was observed. This is indicative of intercalation of starch glycerol moiety along with Fe^{+2}in to the base space of Mt, the layer structure of Mt is maintained.

The diffraction peak at 8.8° had been attributed to the presence of small quantities of elite in the sample.

FTIR Spectrometric Studies

FTIR spectrum of all samples were recorded in the KBr matrix at 25 °C using Perkin Elmer Spectrum BX FTIR spectrometer in the wave number ranged from 4000 to 600 cm^{-1}. Each spectrum is an average of 64sets of data recorded at a 4 cm^{-1} resolution. The FTIR spectrum of Mt and Mt Fe complex has been represented (figure 2). In the spectrum (A), the vibration band at 3624 cm^{-1}, 3410 $cm^{-1}$and 3084 cm^{-1} has been assigned to the O-H stretching vibration of structured water, H-O-H stretching vibration of interlayer water and H-O-H stretching vibration of adsorbed water respectively. The band located at 2500 $cm^{-1}$is a combination band originating from two vibration frequencies of water (surface and/or interlayer water). The vibrational band at 1636cm^{-1} is due to the H-O-H bending vibration of the surface and/or interlayer water (15-16). The stretching vibration of Si-O is observed as a strong absorption band at 1044 cm^{-1}. In the spectrum (B), the disappearance of broad band centered around3084 cm^{-1} and 2500 cm^{-1} is due to the adsorption of Fe^{+2} on the surface of Mt. On the basis of the sharp band at 3326 cm^{-1} and 1636cm^{-1} it can be suggested that Fe^{+2} is also present in the interlayer region as a hydrated ion (17), this is also in support with the XRD data.

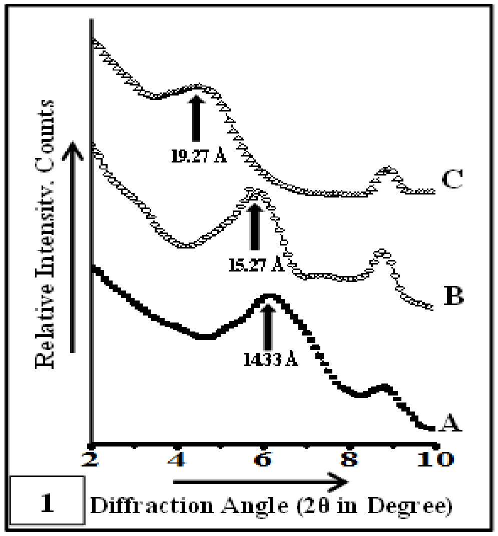

Figure 1: XRD Pattern of: (A) Mt, (B) Mt Fe Complex and (C) Fe^{+2} Mt starch glycerol composite film

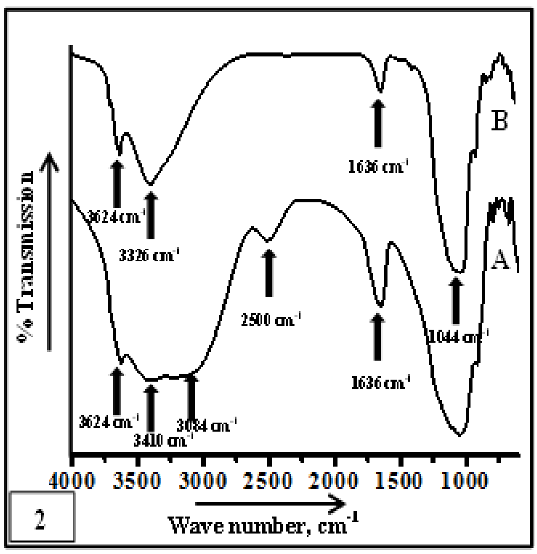

Figure 2: FTIR Spectrum of: (A) Mt, (B) Mt Fe Complex

Thermo Gravimetric Studies

Thermo gravimetric analysis was performed using TGA 2050, Perkin Elmer instrument. A thermo groan of samples was recorded from 30 °C to 850 °C in a nitrogen environment.

The thermo gram of Mt shows three stage weight loss, 12% weight loss at 75°Cis due to the loss of surface adsorbed water, 04% weight loss from 165 °C to 300 °C is due to the loss of interlayer water and 02% weight loss at 605 °C and 726 °C is due to the loss of structural water (figure 3A).

Thermo gram of Fe containing starch glycerol film indicates two weight losses centered around 182 °C and 261°C and are due to the evaporation of glycerol and decomposition of starch involving elimination of poly hydroxyl groups respectively (figure 3B) (21).

The thermo gram of Fe containing Mt starch glycerol composite film is associated with an asymmetric peak centered around 252 °C and is due to glycerol evaporation (appearing as a shoulder at the lower temperature) and starch decomposition (12).

Since, starch and glycerol both are intercalated into the Mt interlayer region by replacing interlayer water therefore; we do not observe weight loss due to interlayer water. We also do not observe loss of structural water (at 605 and 726°C) due to the stabilization of OH groups through interaction with starch.

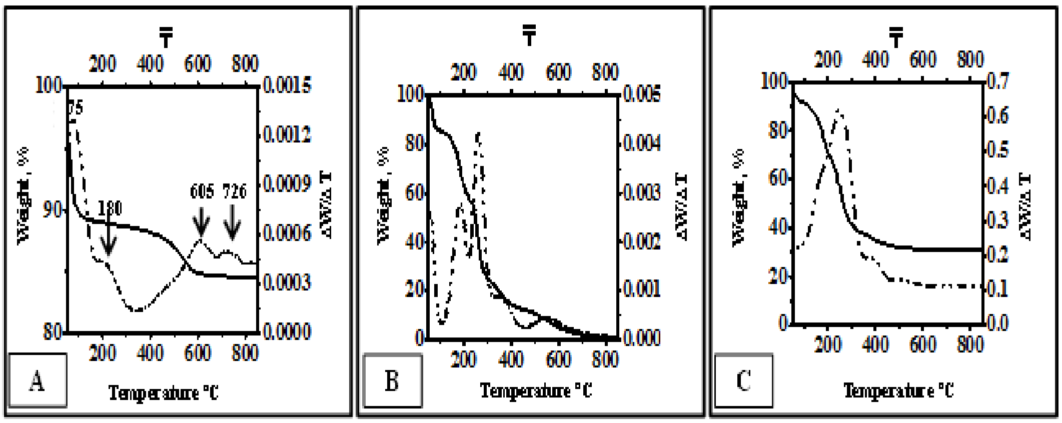

Figure 3: Thermogram of: (A) Mt, (B) Fe Containing Starch Glycerol, and Film and (C) Fe Containing Mt Starch Glycerol Composite Film TGA(——) and DTG Curves(–·)

Microscopic Studies

Scanning electron microscopic (SEM) analysis with energy dispersive X-ray analysis facility was performed using JEOL, JSM-6610LV instrument and high resolution transmission electron microscopic (HRTEM) analysis with energy dispersive X-ray analysis facility was performed using TECNAI G2 T30 U-TWIN model. SEM and HRTEM images of Fe^{+2}starch glycerol film, Fe^{+2} Mt starch glycerol composite film and Fe^{+2} intercalated Mt starch glycerol composite film are shown in figure 4 to 7.

In case of Fe^{+2} starch glycerol film SEM images indicates uniform distribution of crystalised $FeSO_4$ saltson the surface with average particle size of3.5µ/350nm (Figure 4 A). In HRTEM images indicate thin film structure and EDX data proves the presence of Fe in the film.

In case of Fe^{+2} Mt starch glycerol composite film SEM images indicates uniform distribution of crystalised $FeSO_4$ salts on the surface with average particle size of 0.95µ/ 95nm (figure 5 A). In HRTEM images indicate uniform particle size and EDX proves the presence of Si, Al, Mg, Ca and Fe in the sample.

In case of Fe^{+2} intercalated Mt starch glycerol composite film SEM images indicates uniform but less prominent distribution of Fe salts that is because of Fe being intercalated into the Mt layer as supported by XRD data. TEM data indicates uniform particles size of about 40nm (figure 7 A) and also indicates the presence of uniform layer structure of clay in each particle (figure 7 B). EDX proves the presence of of Si, Al, Mg, Ca and Fe in the sample.

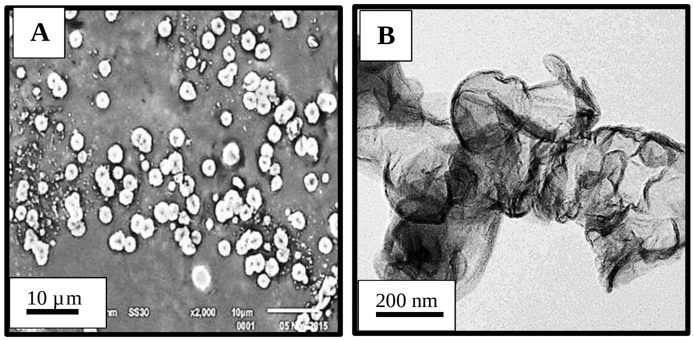

Figure 4: (A) SEM Image, (B) HRTEM Image of Fe^{+2} Starch Glycerol Film

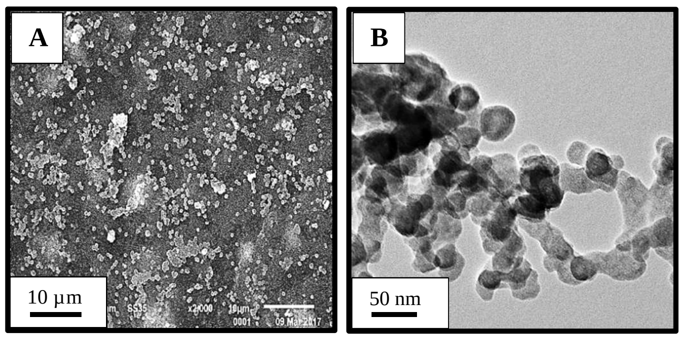

Figure 5: (A) SEM Image, (B) HRTEM Image of Fe^{+2}Mt Starch Glycerol Film

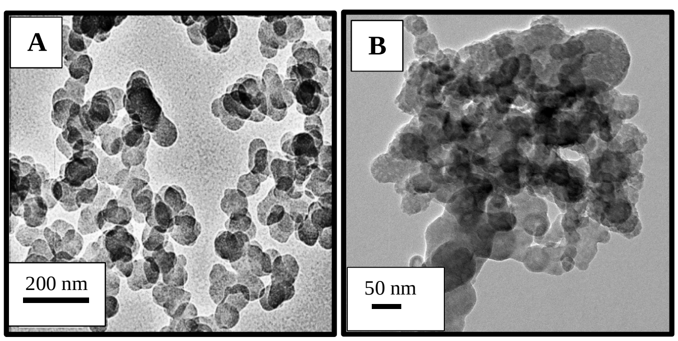

Figure 6: HRTEM Image of Mt (Fe^{+2} Intercalated) Starch Glycerol Film image (A and B)

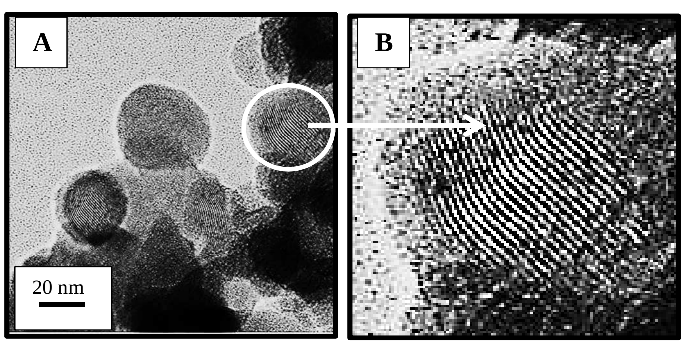

Figure 7: (A) HRTEM Image of Mt (Fe^{+2} Intercalated) Starch Glycerol Film,
(B) Magnified Image Indicating Mt layers

Barrier Properties of Synthesised Films

Barrier property is the ability of a material to transport an object/s from high density side to low density side (22). Gases or water vapor enters through the surface of the material from the higher density side, after diffusing inside it deserves on the low density side as depicted in figure 8. Barrier properties are very important parameters in deciding the suitability of a material for coating seed/s. Therefore, these properties, as discussed below, were experimentally evaluated for all the synthesized films.

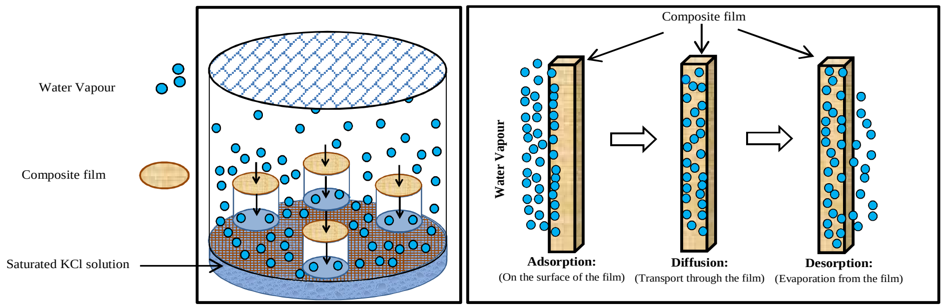

Figure 8: Diffusion of Water though Composite Film

- **Swelling Capacity and Pore Volume**

Swelling capacity and pore volume of films were evaluated by immersing (known weight) of each film in 50ml of double distilled in a beaker, kept at 25°C. Each film was taken out of their respective container and was weighed after removing excess water from the surface of the film; this process was repeated every three hours till a constant weight was attained. From these data percent swelling and pore volume were calculated for each film using the reported procedure (23). Swelling capacity was observed to be 87% and 76% for starch glycerol and Mt starch glycerol composite film respectively. The Mt starch glycerol composite film shows a slower rate of swelling as compared to the starch glycerol film. The pore volume was found to be 56% and 49% for the starch glycerol and Mt starch glycerol composite film.

- **Water Vapour Transmission Rate (WVTR):**

Water vapour transmission is the property of polymeric film, under specific humidity in which water vapor flow in unit time through unit area of polymeric film. Starch glycerol and Mt starch glycerol composite film were kept in a desiccator at 25°C, inside desiccators where relative humidity was maintained at 85 %by using saturated solution of KCl. The films were fixed on the wide mouth bottle (figure 8) and were placed in the desiccator. Each container was taken out from the desiccator and was weighed; this process was repeated every three hours till a constant weight was attained. From these data WVTR was calculated for each film using the reported procedure (23-26). The Mt starch glycerol composite film shows slower WVTR as compared to the starch glycerol film.

- **Solubility**

Solubility is a physical property related to the ability of material to dissolve in water, it is an important parameter for the seed coating material/s so that when discharged into the soil it can disintegrate/decompose naturally. Each film was immersed in a beaker containing 50 ml of double distilled water and was kept at 25°C for 24 h on a mechanical stirrer. Starch glycerol film and Mt Starch glycerol composite film were 50% soluble over a period of 13 days and 22 daysrespectively.

In-Vitro Release of Micronutrient, Iron

The best composition (F-03, table1) was utilized for the synthesis of Fe^{+2} containing composite films. Micronutrient (Fe^{+2}) was varied from 500-3000 µg. The composition providing maximum release of Fe^{+2} (F-Fe- 05, table 2) was utilized for the synthesis of Mt containing composite films, Mt was varied from 20-100 mg. Addition of Mt extends the release of Fe^{+2}best result was obtained in the case of sample number F: Fe – 09 and F: Fe – 10, release was found to be over a period of 15 days. Uniform film was formed in case of F: Fe – 09. Therefore, F: Fe – 09 was used as seed coating material.

Table2: Cumulative Release of Iron from Synthesized Starch Glycerol and Mt Starch Glycerol Composite Film

Film Code	Fe^{+2}, µg	Mt, mg	Cumulative Release of Fe^{+2}		Release Period
			%	µg	
F: Fe – 01	500	-----	91.36	456.80	05 Days
F: Fe – 02	1000	-----	91.23	912.30	05 Days
F: Fe – 03	1500	-----	87.39	1310.85	09 Days
F: Fe – 04	2000	-----	87.40	1748.00	09 Days
F: Fe – 05	2500	-----	87.42	2185.50	09 Days
F: Fe – 06	2500	20	76.34	1908.50	10 Days
F: Fe – 07	2500	40	76.27	1906.75	11 Days
F: Fe – 08	2500	60	76.14	1903.50	13 Days
F: Fe – 09	2500	80	75.50	1887.50	15 Days
F: Fe – 10	2500	100	75.21	1880.00	15 Days

Dissolution apparatus (DISSO 8000, Lab India, India) was utilized for the study of *in-vitro* release of Fe^{+2} from the synthesized starch glycerol and clay starch glycerol composite films. 5.0 cm diameter circular film was kept in the USP-1 40 mesh size net basket and the film containing basket was immersed into 500 ml dissolution medium (aqueous soil extract) contained in a bowl. The solution was kept on constant stirring (20 RPM) and the entire system was maintained at 25°C.

At an interval of 24 hour 10 ml of the dissolution medium was withdrawn and was replenished with fresh 10 ml of dissolution medium this process was repeated several times till no further release of Fe^{+2} was observed. Concentration of Fe^{+2}in each fraction were estimated by flame atomic absorption spectrometry.

Percentage cumulative release of Fe^{+2}in each case was plotted as a function of time (figure 9). The release of $Fe^{+2}$from starch glycerol films containing clay (Montmorillonite, Bentonite and Vermiculite) appears to be more extended in time than starch glycerol film, among the three clays Mt provides maximum extended release of micronutrient therefore, this composition was utilized for coating seeds requiring Fe^{+2}in the early stage of plant growth.

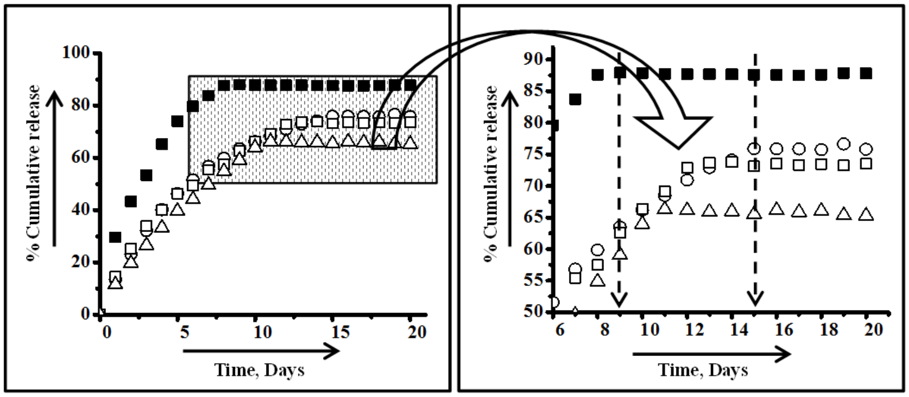

Figure 9: Cumulative Release Behaviour of Fe^{+2}:
■ - Starch Glycerol Film, ○ - Mt Starch Glycerol Composite Film
□ - Bt Starch Glycerol Composite Film and △ - Vt Starch Glycerol Composite Film

Application of Iron Containing Clay Polymer Composite Film

The Mt starch glycerol composite film was utilized for coating of seeds (wheat) requiring Fe^{+2} in the early stage of plant growth. These coated seeds can potentially overcome problems of insufficient fertilization as well as the possibility of growing plants, even in the areas having soil deficient in micronutrient, Fe^{+2}. Micronutrient will be within the biodegradable film, on the surface of the seed and will release in an extended manner in the vicinity of the sown seeds. This method will ensure the availability of micronutrients to the seedling at the time of growth for a required duration of time. It will also substantially reduce the cost of providing micronutrients to the seedlings. Wheat seeds have been coated with Fe^{+2} containing Mt starch glycerol composite film and have been shown in normal and deficient soil, results indicate better crop production in case of coated seeds. This work will be published separately.

CONCLUSIONS

In order to provide micronutrient to the seedlings and growing plants in the early stage of growth (first three to four weeks, 6), two types of environmentally friendly biodegradable films containing micronutrient have been synthesized with the aim to provide micronutrients to plants in their early stage of growth in an extended release manner. Starch and glycerol have been used to develop a polymeric material, the effect of various naturally occurring clays (Montmorillonite, Bentonite and Vermiculite) on the quality of polymeric film and *in-vitro* release behavior of Fe^{+2} from these films have been evaluated. XPS data indicate the presence of Fe^{+2} in all films. Addition of Mt reduces the crystal size substantially, from 350nm to 95nm while maintaining uniform distribution in the film. However, when Fe^{+2}intercalated Mt was used as a source of iron the SEM images do not show the presence of $FeSO_4$ crystals in the film instead, uniform particle size of 40 mm with Mt layer structure was visible in each particle. EDX of all samples containing Mt show the presence of Si, Al and Cu along with Fe. On the basis of information obtained from the XRD, FTIR spectrometry, Zeta potential and TEM images it can be concluded that Fe^{+2} is mostly present in between the clay platelets.

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REFERENCES

1. *Zdenkorengel et al. (1995), Importance of seed Zn content for wheat growth on Zn- deficient soil, plant and soil, 173, 259-266.*
2. *https://en.wikipedia.org/wiki/Plant nutrition*
3. *http://www.ncagr.gov/cyber/kidswrld/plant/nutrient.htm*
4. *Hamid Reza Mobasser et.al. (2014), Effect of application elements, water stress and variety on nutrients of grain wheat in Zahak region, Journal of biodiversity and environmental sciences, 5(1), 105-110.*
5. *M. Farooq et.al. (2012), Micronutrient application through seed treatments - a review, Journal of soil science and plant nutrition, 12 (1), 125-142.*
6. *Jackie S Mote et.al. (2007), A polymer based seed coating, WO 2007103076 Al. Application number PCT/US2007/005146.*
7. *https://en.wikipedia.org/wiki/Micronutrient*
8. *Theodore H Tulchinsky et.al. (2009), Micronutrient deficiency conditions: global health, Public health reviews, 32(1), 243-255.*
9. *Rout et.al. (2015) Role of iron in plant growth and metabolism, Reviews in Agricultural Science, 3, 1-24.*
10. *Shanyn Hosier et al. (1999), Guide to Symptoms of plant nutrient deficiencies,*
11. *PiyapornKampeerapappun et.al. (2007), Preparation of cassava starch/montmorillonite composite film, Carbohydrate polymers 67, 155–163.*
12. *Xiaozhi Tanget. al. (2008) Barrier and mechanical properties of starch-clay nanocomposite films, Journal of cereal chemistry.85 (3), 433-439.*
13. *Fauze A. Aouadaaet. al. (2011), New strategies in the preparation of exfoliated thermoplastic starch–montmorillonite nanocomposites, Industrial crops and products 34, 1502– 1508.*
14. *Dipti Rani Mishra et.al. (2011), Phisico-chemical properies of environmental friendly starch-mmtnanocomposites for film making, International journal of plant, animal and environmental sciences, 1, (2),134-144.*
15. *S.Y. A. Shin (2007). Ph.D. Thesis, In-Situ Polymerization and Characterization of Polyethylene-ClayNanocomposites*
16. *P. Djomgoue et al (2013). FT-IR Spectroscopy Applied for Surface Clays Characterization, Journal of Surface Engineered Materials and Advanced Technology, 3, 275-282*
17. *Neeraj kumariet. al. (2016), An integrated approch for the removal of indigo carmine dye from its aqueous solution and the synthesis of nano pigment, International Journal of environment, Ecology, Family and urban studies, 6(5), 7-16.*
18. *Kovo G. Akpomie et al. (2015). Potential of a low-cost bentonite for heavy metal abstraction from binary component system, Journal of Basic and Applied Sciences, 4 (1), 1-13.*
19. *Seemaet al (2013). Mmt-PlgaNanocompositesAs An Oral And Controlled Release Carrier For 5-Fluorouracil: A Novel Approach, International Journal of Pharmacy and Pharmaceutical Sciences, 5(2), 332-341.*
20. *Seemaet. al. (2014). OrganoclayPluronic F68 – Montmorillonite, As A Sustained Release Drug Delivery Vehicle For Propranolol Hydrochloride, European Chemical Bulletin, 3(6), 593-604.*
21. *M. Kauret. al. (2014). In Vitro Release Of Sodium Diclofenac From Poloxamer 188 Modified Montmorillonite As An Oral Drug Delivery Vehicle, International Journal of Pharmacy and Pharmaceutical Sciences, 6 (5), 100-110.*

22. *Viviana P. Cyrasalet. al. (2006). Relationship Between Structure and Properties of Modified Potato Starch Biodegradable Films, Journal of Applied Polymer Science, 101, 4313– 4319.*

23. *http://www.netparkonline.in/tech/what-is-barrier-property-of-materials/*

24. *SLAVUTSKY et. al., Water barrier properties of starch-clay nanocomposite films, Braz. J. Food Techno, Campinas, 15(3), 208-218.*

25. *Arham, R. et. al (2016)., Physical and mechanical properties of agar based edible film with glycerol plasticizer, International Food Research Journal 23(4), 1669-1675.*

26. *M.L. Sanyang.et. al., Effect of glycerol and sorbitol plasticizers on physical and thermal properties of sugar palm starch based films, Recent Advances in Environment, Ecosystems and Development, 978-1-61804-301-6*